Combined Assessment of TGF-beta-1 and Alpha-Fetoprotein Values Improves Specificity in the Diagnosis of Hepatocellular Carcinoma and Other Chronic Liver Diseases in Malaysia

M Y Yasmin Anum, PhD*, M L Looi, Phd*, A H Nor Aini, MMedSc*, I Merican, FRCP**, A Wahidah, MPath***, A H Mohd Radzi, MRCP****, A Nor Azizah, BSc***** , N H Othman, MPath******

*Department of Biochemistry, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Center, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, **Ministry of Health, Putra Jaya, Selangor, ***Hospital Tengku Ampuan Rahimah, Klang, Selangor, ****Hospital Sultanah Bahiyah, Alor Setar, Kedah, *****School of Allied Health and ******Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, 16150 Kelantan, Malaysia

SUMMARY

Transforming growth factor beta-1 (TGF-β-1) is a multifunctional cytokine involved in the regulation of growth and differentiation of both normal and transformed cells. The main aim of this study was to determine whether TGF-β-1 or alpha fetoprotein (AFP) or the combination of the two is a better indicator for hepatocellularcarcinoma (HCC). Serum TGF-β-1 and AFP were measured by ELISA in 40 healthy subjects, 23 patients with hepatocellular carcinoma (HCC), 70 patients with hepatitis B, 26 patients with hepatitis C and 16 patients with liver cirrhosis (LC). Patients with liver diseases showed significantly higher serum TGF-β-1 values (> 3 fold) compared to control subjects. As for serum AFP, significant elevation was only observed for HCC cases. Serum TGF-β-1 exhibited higher percent sensitivity compared to serum AFP in all liver diseases. Combination of serum TGF-β-1 and AFP increased specificities in all cases studied. In conclusion, serum TGF-β-1 is a more sensitive marker for HCC when compared to serum AFP and its specificity is increased when combined with serum AFP.

KEY WORDS:
AFP, TGF-β-1, Hepatocelllular carcinoma, Tumour marker

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most prevalent cancers in the world[1]. In Malaysia it is the 13th and 18th most common cancers affecting the male and female population respectively[2]. It is well documented that hepatitis B or C infections are major risk factors of HCC, and that HCC and cirrhosis frequently coexist within the same liver[3-6]. The strong association of hepatotropic viral infections and HCC with coexistent cirrhosis suggests that the common pathway for hepatocarcinogenesis may be chronic hepatic injury due to repeated and persistent HBV or HCV infections that finally results in liver cirrhosis and HCC[7,8]. Most patients with HCC are diagnosed at a late stage, making prognosis of HCC very poor with five year survival rate of less than 5%.   Thus effective screening strategies are very critical which include a combination of ultrasound and molecular marker(s) such as α-fetoprotein (AFP)[9].   AFP, however is a marker with poor sensitivity and specificity whereby up to 30-40% of HCC have normal AFP levels.   It may also be increased in non-malignant, chronic liver diseases and other malignancies such as germ cell tumours of the testis and ovary[10-12].   Recently AFP-L3, a variant of AFP is shown to be more specific for HCC than total AFP[13].   Other proteins that have been shown to be potential tumour markers of HCC were prealbumin[14] des-gamma-carboxy prothrombin (DCP)[15-18] γ-glutamyl transferase (GGT)[16-19] and transforming growth factor beta-1(TGF-β-1)[20-22].

TGF-β is a family of disulfide-linked polypeptides with a molecular weight of 25kD. There are three isoforms of TGF-β expressed in mammals; TGF-β-1, TGF-β-2 and TGF-β-3[23]. Understanding the mechanism of TGF-β has been the focus of recent studies in unraveling the pathogenesis of many human cancers[23].   TGF-β-1 is the predominant and universally expressed isoform found in human liver. It has been implicated to play a role as a potent inhibitor of both normal and neoplastic rat hepatocyte proliferation as well as in the development of liver fibrosis[24-26]. TGF-β-1 has also been found to be elevated in serum, urine and tissues of patients with HCC[27].   Moreover, TGF-β-1 was elevated in 25% of AFP-negative patients with HCC[28]. The over expression of hepatic TGF-β-1 was found in HCC tissues and correlated well with carcinogenesis, progression and prognosis of HCC[29,30]. Thus, all these studies implicated that TGF-β-1 may be a new generation of tumour marker for HCC. In this study, we determined serum levels of TGF-β-1 and AFP in patients with HCC and non malignant chronic liver disease such as hepatitis B and C infections and liver cirrhosis (LC) to evaluate whether serum TGF-β-1 is a sensitive and useful molecular marker for the diagnosis of HCC.

MATERIALS AND METHODS
Patients

One hundred and thirty five patients with chronic liver disease (16 liver cirrhosis; 70 hepatitis B; 26 hepatitis C and 23 HCC), aged between 18 and 79 years were included in the study. Patients were from three hospitals, Hospital Universiti

Sains Malaysia, Kubang Kerian, Kelantan, Hospital Kuala Lumpur and Hospital Kuala Terengganu, Malaysia. Forty healthy subjects aged between 18-80 years were included in the study as the control group. They were negative for HbsAg and anti-HCV antibody and showed normal liver function. All volunteers and patients gave written informed consent to the procedures, which were approved by the various hospitals ethical committee, and the ethical committee of Medical School Universiti Sains Malaysia, Kubang Kerian, Kelantan. The study was conducted over a 4-year-period (1997-2000) funded by the IRPA 7th Malaysia Plan.

Serum AFP determination
Enzynost AFP ELISA Micro kits were purchased from Behring (Behringwerke AG, Marburg, Germany). One hundred microlitre of buffer solution (Phosphate/citrate buffer, 100 mmol/L) was pipetted into appropriate anti-human AFP coated test wells, followed by addition of 20 µl each of standards, serum samples and/or control into respective wells in duplicates. The test wells were incubated at 37°C for 1 hour. After washings with phosphate buffer solution containing 0.5% Tween 20, monoclonal mouse anti-human AFP/Peroxidase conjugate was added to each well and incubated at 37°C for 1 hour. After three washings, chromogen peroxidase solution (0.3 g/L hydrogen peroxide in citrate phosphate buffer solution mixed with o-phenylenediamine hydrochloride, 0.4 mg/ml in citrate phosphate buffer) was added to each well and incubated for 30 min in the dark. The reaction was terminated with stopping solution (100 µL of 0.25 M sulphuric acid in each well) which produced yellow colour. Absorbance was read at 492 nm using Dynatech MR5000 microplate reader (*Dynatech* Laboratories, Chantilly, Va. USA). The coefficient of variation (CV) within the series for AFP was found to be between 2.1% and 5.1% for concentrations in the range from 4 to 180 IU/ml (intra assay CV) and the day to day coefficient (inter assay CV) was found to be between 3.9% and 8.0%.

Serum TGF-β-1 determination
TGF-β-1 ELISA kits were purchased from GENZYME (Genzyme Corp., Cambridge, MA, USA). Standards, serum samples and controls were diluted and acidified using 1 M hydrochloric acid for 1 hour at 2-8°C. Acidified samples and standards were neutralized to pH7.0-7.4 with 1 M sodium hydroxide. 100 µl of activated standards and samples were added into appropriate monoclonal mouse anti-human TGF-β-1 coated test wells in duplicate and incubated at 37°C for 1 hour. The contents of the test wells were then aspirated and rinsed with wash reagent (detergent solution with 0.02% thimerosal as preservative), repeatedly three times. One hundred microlitre of anti TGF-β-1/Horse Radish Peroxidase-conjugate was added into each test well and incubated at 37°C for 1 hour. The contents of test wells were aspirated and washed again as previously. The substrate reagent (<2 N tetramethylbenzidine in < 40% methanol and 0.03% hydrogen peroxide in buffered solution) was added into each test well, and incubated at room temperature for 20 minutes to produce a blue colour reaction. Finally the reaction was

terminated by adding the stop solution (< 2 N mixture of acid) and the absorbance was read at 450nm within 30 minutes, using Dynatech MR5000 microplate reader (*Dynatech* Laboratories, Chantilly, Va. USA). The CVs for 3 samples diluents preparations containing low, medium and high levels of activated TGF-β-1 were assayed using TGF-β-1 kit for a total of 24 determinations giving an intra-assay CVs of 4.65%, 5.2% and 4.8% respectively, and an inter assay CVs of 11.0%, 5.2% and 4.8% respectively.

Statistical analysis
Statistical significance was taken at p<0.05. All data analysis was performed using SPSS version 11.0.

RESULTS
Table I showed that mean values (mean ± SD) for serum TGF-β-1 and AFP in healthy subjects were 14.35 ± 8.76 ng/ml and 2.6 + 1.9 IU/ml respectively. The mean values of serum TGF-β-1 and AFP in HCC patients were 64.33 ± 33.68 ng/ml and 81.29 ± 164.27 IU/ml respectively. (The standard deviations were large for serum AFP due to a wide variation of AFP values in HCC patients, ranging from 0.06 to 561.25 IU/ml). Serum TGF-β-1 levels were significantly higher (p< 0.001) in HCC, liver cirrhosis, hepatitis B and hepatitis C than control subjects. However, serum AFP levels were only significantly higher (p< 0.05) in HCC patients compared to control subjects but not in other chronic liver diseases.

Table II showed the correlations between serum TGF-β-1 and AFP values in HCC and chronic liver diseases. No correlation was found between these two serum markers in all cases.

Using the gold standard 2 x 2 table with cut off values of 8.4 IU/ml for AFP and 31.87 ng/ml for TGF-β-1 (mean ± 2 SD) percent sensitivity of TGF-β-1 were generally higher compared to AFP for all cases of liver disease (Table III): 78.3% versus 52.2% for HCC, 50.0% versus 37.5% for LC , 77.1% versus 21.4% for hepatitis B, and 65.4% versus 11.5% for hepatitis C respectively. Percent specificities of serum TGF-β-1 for all cases were generally lower compared to serum AFP: 29.5% versus 78.6% for HCC, 25.2% versus 74.8% for LC, 33.8% versus 67.7% for hepatitis B, and 26.6% versus 69.7% for hepatitis C respectively. When both tests were combined an improvement of specificities was observed; 86.6% for HCC, 76.8% for hepatitis B, 82.1% for hepatitis C, 84% for LC. However, percent sensitivities declined when both tests were combined. Table III also showed the negative and positive predictive values (NPVs, PPVs) of serum markers TGF-β-1 and AFP. Except for hepatitis B, PPVs were generally lower for both serum markers in all liver disease. However, NPVs were > 75% for both serum markers in all liver disease with an exception of hepatitis B. For HCC, positive and negative likelihood ratios (PLR, NLR) for serum AFP, and combination of AFP and TGF-β-1 showed small changes but there were no significant changes observed in the PLR and NLR with other liver diseases.

Table I: Serum TGF-β-1 and AFP in HCC and other chronic liver diseases

	AFP (IU/ml)	TGF-β-1 (ng/ml)
Control (N = 40)	2.6 ± 1.9	14.35 ± 8.76
HCC (N = 23)	81.29 ± 164.27*	64.33 ± 33.68*
LC (N = 16)	32.42 ± 77.05	61.16 ± 51.6*
Hepatitis B (N = 70)	66.84 ± 498. 73	65.31 ± 50.11*
Hepatitis C (N = 26)	54.76 ± 314.77	72.21 ± 71.21*

Data represents mean ± S.D
* = significant when compared to control subjects , p <0.05
 HCC, hepatocellular carcinoma; AFP, alpha feto protein; TGF-β-1, transforming growth factor beta-1; LC, liver cirrhosis

Table II: Correlation between serum TGF-β-1 and AFP in Liver diseases

Diseases	Serum markers	AFP	TGF-β-1
HCC	AFP	1.00	-0.15
	TGF-β-1	-0.15	1.00
LC	AFP	1.00	-0.14
	TGF-β-1	-0.14	1.00
Hepatitis B	AFP	1.00	-0.03
	TGF-β1	-0.03	1.00
Hepatitis C	AFP	1.00	-0.08
	TGFβ1	-0.08	1.00

Table III: Percent sensitivity, specificity, positive and negative predictive values (PPV, NPV), positive and negative likelihood ratio (PLR, NLR) for serum TGF-β-1 and AFP in HCC and other chronic liver diseases

Diseases	Serum markers	Sensitivity (%) †	Specificity (%) ‡	PPV (%) §	NPV (%) ¶	PLR ¥	NLR ∈
HCC (N = 23)	AFP	52.2	78.6	33.3	88.9	2.4	1.6
	TGFβ1	78.3	29.5	18.6	86.8	1.1	1.4
	AFP + TGFβ1	30.4	86.6	31.8	85.8	2.3	1.2
LC (N = 16)	AFP	37.5	74.8	16.7	89.9	1.5	1.2
	TGFβ1	50.0	25.2	8.2	78.9	0.7	0.5
	AFP + TGFβ1	18.8	84.0	13.6	88.5	1.2	1.0
Hepatitis B (N = 70)	AFP	21.4	67.7	41.7	44.4	0.7	0.9
	TGFβ1	77.1	33.8	55.7	57.9	1.2	1.5
	AFP + TGFβ -1	11.4	76.8	40.9	38.1	0.5	0.9
Hepatitis C (N = 26)	AFP	11.5	69.7	8.3	76.8	0.4	0.8
	TGFβ1	65.4	26.6	17.5	76.3	0.9	0.8
	AFP + TGFβ1	10.3	82.1	13.6	77.0	0.6	0.9

†Sensitivity = TP / (TP + FN) X 100 %; ‡ Specificity = TN / (TN + FP) X 100 %; §PPV = TP / (TP + FP) X 100 %; ¶ NPV = TN / (TN + FN) X 100 %; ¥ PLR = Sensitivity / (1- specificity); ∈NLR = Specificity / (1- sensitivity); TP = true positive, FN = false negative, PPV = Predictive positive values, NPV = negative predictive values, PLR = positive likelihood ratio, NLR = negative likelihood ratio

DISCUSSION

HCC is one of the most prevalent causes of death in the world and majority of HCC develop from cirrhotic livers and livers that have underwent repeated HBV and HCV infections. Serum AFP is the most widely used oncomarker for detection of HCC and chronic liver disease[28,31].   However, several observations have shown AFP to be unreliable if used alone in diagnosing HCC[10,14,16,32].   Combination of serum TGF-β-1[21], γ glutamyl transferase (GGT II) and PIVKA II [15,16] has been shown to improve the sensitivity and specificity in diagnosing HCC. Although increased levels of TGF-β-1 has been found in the serum of patients with several cancers such as breast,[33] colorectal,[34] lung, [35] and liver, [21] a diagnostic role for elevated TGF-β-1 levels has not been established yet. We have found in our study that the sensitivity of serum marker TGF-β-1 surpassed that of AFP in the diagnosis of HCC and other non malignant liver diseases.   Our finding is similar to Song et al.,[21] who demonstrated that   serum level of TGF-β-1 was a more sensitive indicator for small HCC than AFP suggesting that measuring serum TGF-β-1 level may be clinically useful for diagnosing liver tumours. In addition, serum TGF-β-1 was more sensitive in detecting HCC compared to other liver diseases. Although serum TGF-β-1 showed lower specificity compared to serum AFP, and with no significant correlation found between both markers, combining the two parameters however improved the specificity of the test.   This was also true as reported by Dong et al.,[36] whereby combining circulating TGF-β-1 and AFP levels raised the detection rate to 97.4% although no significant correlation was found between the two markers.   Specificity and sensitivity alone may not help clinicians in diagnosing HCC, however positive and negative predictive values of test of interest should be included.   The positive predictive value (PPV) predicts the probability that a person is having the disease given a positive

test result, while negative predictive value (NPV) predicts the probability that a person does not have the disease given a negative test. Predictive values however are influenced by prevalence of a disease, the higher the prevalence of a particular disease, the higher positive and negative predictive values of a test. In Malaysia, the incidence of HCC is 4.5%,[2] and with this low incidence we found low PPV's for both TGF-β-1 and AFP in all cases of liver diseases. However, NPV's for both serum TGF-β-1 and AFP were high in HCC, showing that the probability of the population who were diagnosed not to have the disease is high. Elevation of TGFβ-1 in chronic liver disease (hepatitis B, C and LC) may reflect the process of fibrogenesis prior to formation of HCC[37,38]. It was postulated that TGF-β may be involved in the proliferation and differentiation of special type of pre-cancerous cells known as oval cells in the early stage of hepatocarcinogenesis into ductular cells which later progress into neoplastic cells of HCC[39]. In human study, oval cells have been sighted and stained positively with M2 pyruvate kinase in LC and HCC cases and their numbers were significantly related to the staging of liver fibrosis[40,41].

Not many studies have been dedicated to the diagnostic role of TGF-β-1 in chronic liver diseases. This study and several others have shown the usefulness of TGF-β-1 as serum indicator of HCC and other chronic liver diseases [6-8,13,42-44]. TGF-β-1 can be a possible marker for hepatic fibrosis progression from chronic liver disease to HCC[42]. In clinical cases where increased activity of TGF-β-1 has been correlated with tumorigenesis, attempts to decrease or abrogate TGF-β-1 signaling by blocking or inhibiting TGF-β-1 binding to its receptors may be used as a therapy for advanced or metastatic disease[23].

The incidence of hepatocellular carcinoma (HCC) is increasing worldwide with cirrhosis being the strongest risk factor for the development of HCC[1,5]. Although HCC screening with AFP and ultrasonography has been recommended for persons with cirrhosis, its level is insensitive for the early detection of HCC, and ultrasonography is expensive and operator dependent. Clearly, there is a need for novel biomarkers for the early detection of HCC. In this study we have shown that combination of the two biomarkers AFP and TGF-β resulted in an increase of the specificity and sensitivity in detecting HCC. However, limitations of the current study and other literatures include inadequate sample size, limited analysis and a scarcity of longitudinal studies evaluating the ability of other biomarkers besides AFP to detect preclinical disease and to correlate levels of biomarkers with severity of HCC. Thus, the future direction of this research would be more appropriate if an association between liver tumour markers and tumour size could be established.

REFERENCES
1. Kew MC. Epidemiology of hepatocellular carcinoma. Toxicol 2002; 35; 181-82.
2. Lim CC, Halimah Y. 2nd Report of National Cancer Registry of Cancer Incidence in Malaysia 2003.
3. Tsai JF, Chang WY, Jeng JE, Ho MS, Lin ZY, Tsai JH. Hepatitis B and C virus infection as risk factors for liver cirrhosis and cirrhotic hepatocellular carcinoma: A case-control study. Liver 1994; 14: 98-102.
4. Tsai JF, Chang WY, Jeng JE, Ho MS, Lin ZY, Tsai JH. Effects of hepatitis C and B viruses infection on the development of hepatocellular carcinoma. J Med Virol 1994; 44: 92-95.
5. Jeffers L. Hepatocellular carcinoma: an emerging problem with hepatitis C. J Natl Med Assoc 2000; 92: 369-71.
6. Tsai JF, Jeng JE, Chang WY et al. Additive effect modification of hepatitis B surface antigen and e antigen on the development of hepatocellular carcinoma. Br J Cancer 1996; 73: 1498-502.
7. Tsai JF, Margolis HS, Jeng JE et al. Circulating immune complexes in chronic hepatitis related to hepatitis C and B viruses infection. Clin Immunol Immunopathol 1995; 75: 39-44.
8. Tsai JF, Margolis HS, Jeng JE et al. Hepatitis B surface antigen and immunoglobulin-specific circulating immune complexes in acute hepatitis B virus infection. Clin Immunol Immunopathol 1996; 80: 278-82.
9. Thompson Coon J, Rogers G, Hewson P et al. Surveillance of cirrhosis for hepatocellular carcinoma: a cost-utility analysis. Br J Cancer 2008; 98: 1166-75.
10. Tsai JF, Chang WY, Jeng JE, Ho MS, Lin ZY, Tsai JH. Frequency of raised AFP level among Chinese patients with hepatocellular carcinoma related to hepatitis B and C. Br J Cancer 1994; 69: 1157-59.
11. Lopez PM, Villanueva A, Llovet JM. Evidence-Based Management of Hepatocellular Carcinoma - An Updated Analysis of Randomized Controlled Trials. Aliment Pharmacol Ther. 2006; 23(11): 1535-47.
12. Spangenberg HC, Thimme R, Blum HE. Serum markers of hepatocellular carcinoma. Seminars in liver disease. 2006; 26: 385-90.
13. Li D, Mallory T, Satomura S. AFP-L3: a new generation of tumour marker for hepatocellular carcinoma. Clin Chim Acta 2001; 313: 15-19.
14. Mohd Yusof YA, Baba A, Salleh MN, Ramankutty M. Prealbumin rather than albumin is a more sensitive indicator of acute liver disease. Malaysian J Pathol 1993; 15: 147-50.
15. Liebman HA. Isolation and characterization of a hepatoma-associated abnormal (des-gamma-carboxy) prothrombin. Cancer Res 1989; 49: 6493-97.
16. Cui R, He J, Zhang F et al. Diagnostic value of protein induced by vitamin K absence (PIVKA II) and hepatoma-specific band of serum gamma-glutamyl transferase (GGT II) as hepatocellular carcinoma markers complementary to alpha-fetoprotein. Br J Cancer 2003; 88: 1878-82.
17. Liebman HA, Furie BC, Tony MJ et al. Des-gamma –carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. N Engl J Med 1984; 310: 1427-31.
18. Marrero JA, Su GL, Wei W et al. Des-gamma carboxyprotrhombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in American patients. Hepatol 2003; 37: 1114-21.
19. Yao DF, Jiang DR, Huang ZW et al. Abnormal expression of hepatoma specific ?-glutamyl transferase and alteration of ?-glutamyl transferase gene methylation status in patients with hepatocellular carcinoma. Cancer 2000; 88: 761-69.
20. Kim HG, Chung YH, Song BC et al. Expression of transforming growth factor beta-1 in chronic hepatitis and hepatocellular carcinoma associated with hepatitis C virus infection. Korean J Intern Med 2000; 15: 165-70.
21. Song B-C, Chung Y-H, Kim JA et al. Transforming growth factor-β1 as a useful serologic marker of small hepatocellular carcinoma. Cancer 2002; 94: 175-80.
22. Mohd Yusof YA, Salleh MN, Abdullah NA. Elevation of serum transforming growth factor (TGF)-beta-1 and reduction of serum pre-albumin (PAB) and its relationship to serum alpha-fetoprotein (AFP) levels in patients with hepatocellular carcinoma. Malaysian J Biochem and Molec Biol 1997; 2: 18-22.
23. Elliott RL, Blobe GC. Role of transforming growth factor beta in human cancer. J Clin Oncology 2005; 23: 2078-93.
24. Cross M, Dexter TM. Growth factors in development, transformation and tumorigenesis. Cell 1991; 64: 271-80.
25. Bedossa P, Paradis V. Transforming growth factor-beta (TGF-beta): A key role in liver fibrogenesis. J Hepatol 1995; 22: Suppl 2: 37-42.
26. Wollenberg GK, Semple E, Quinn BA, Hayes MA. Inhibition of proliferation of normal, preneoplastic, and neoplastic rat hepatocytes by transforming growth factor-beta. Cancer Res 1987; 47: 6595-99.
27. Sacco R, Leuci D, Tortorella C et al. Transforming growth factor beta-1 and soluble Fas serum levels in hepatocellular carcinoma. Cytokine 2000; 12: 811-14.
28. Liaw YF, Tai DI, Chu CM et al. Early detection of hepatocellular carcinoma in patients with chronic type B hepatitis. A prospective study. Gasteroenterol 1986; 90: 263-7.

29. Bissell DM, Roulot D, George J. Transforming growth factor beta and the liver. Hepatol 2001; 34: 859-67.

30. Okumoto K, Hattori E, Tamura K *et al.* Possible contribution of circulating transforming growth factor-beta 1 to immunity and prognosis in unresectable hepatocellular carcinoma. Liver Int 2004; 24: 21-28.

31. Colombo M, de Franchis R, Del Ninno E *et al.* Hepatocellular carcinoma in Italian patients with cirrhosis. N Eng J Med 1991; 325: 675-80.

32. Pateron D, Ganne N, Trinchet JC *et al.* Prospective study of screening for hepatocellular carcinoma in Caucasian patients with cirrhosis. J Hepatol 1994; 20: 65-71.

33. Gorsch SM, Memoli VA, Stukel TA, Gold LI, Arrick BA. Immunohistochemical staining for transforming growth factor beta 1 associates with disease progression in human breast cancer. Cancer Res 1992; 52: 6949-52.

34. Shim KS, Kim KH, Han WS, Park EB. Elevated serum levels of transforming growth factor-beta 1 in patients with colorectal carcinoma: Its association with tumor progression and its significant decrease after curative surgical resection. Cancer 1999; 85: 554-61.

35. Barthelemy-Brichant N, David JL, Bosquee L *et al.* Increased TGF-beta 1 plasma level in patients with lung cancer: potential mechanisms. Eur J Clin Invest 2002; 32: 193-98.

36. Dong ZZ, Yao DF, Yao M *et al.* Clinical impact of plasma TGF β1 and circulating TGF β1mRNA in diagnosis of hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int 2008; 7: 288-95.

37. Castilla A, Prieto J, Fausto N. Transforming growth factor β1 and α in chronic liver disease:effects of interferon alpha therapy. N Eng J Med 1991; 324: 934-40.

38. Bedossa P, Peltier E, Teris B, Franco D, Poynard T. Transforming growth factor beta-1 (TGF β1) and TGF β1receptors in normal, cirrhotic, and neoplastic human livers. Hepatol 1995; 21: 760-65.

39. Hsia CC, Evarts RP, Nakatsuka H, Marsden ER, Thorgeisson SS. Occurrence of oval-type cells in hepatitis B virus-associated human hepatocarcinogenesis. Hepatol 1992; 16: 1327-33.

40. Lowes KN, Brennan BA, Yeoh GC, Olynyk JK. Oval cell numbers in human chronic liver diseases are directly related to disease severity. Amer J of Pathol 1999; 154: 537-40.

41. Dekai Q, Xiong M, Yansken P, Xiaoyu C, Yao S. Localization and quantitative study of hepatic oval cells in patients with chronic liver diseases: a pathological analysis of 29 liver specimens. Chinese J Digestive Disease 2002; 2: 78-82.

42. Flisiak R, Pytel-Krolczuk B, Prokopowicz D. Circulating transforming growth factor β1 as an indicator of hepatic function impairment in liver cirrhosis. Cytokine 2000; 12: 677-81.

43. Shirai Y, Kawata S, Tamura S *et al.* Plasma transforming growth factor beta-1 in patient with hepatocellular carcinoma, comparison with chronic liver disease. Cancer 1994; 73: 2275-78.

44. Ito N, Kawata S, Tamura S *et al.* Elevated levels of TGF-beta-1 messenger RNA and its polypeptide in human hepatocellular carcinoma. Cancer Res 1991; 51: 4080-83.